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Common variants associated with blood lipid levels do not affect carotid plaque composition

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ABSTRACT

Introduction: Although plasma lipid levels are known to influence the risk of cardiovascular disease (CVD), little is known about their effect on atherosclerotic plaque composition. To date, large-scale genome-wide association studies have identified 157 common single-nucleotide polymorphisms (SNPs) that influence plasma lipid levels, providing a powerful tool to investigate the effect of plasma lipid levels on atherosclerotic plaque composition.

Methods: In this study, we included 1443 carotid endarterectomy patients from the Athero-Express Biobank Study with genotype data. Plasma concentrations of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglycerides (TG) were determined at the time of endarterectomy. Atherosclerotic plaques, obtained during surgery, were histologically examined. For all patients, we calculated weighted genetic burden scores (GBS) for all lipid traits on the basis of the available genotype data. Plasma lipid levels and GBS were tested for association with 7 histological features using linear and logistic regression models.

Results: All GBS were associated with their respective plasma lipid concentrations ($p_{HDL\text{-}C} = 2.4 \times 10^{-14}$, $p_{LDL\text{-}C} = 0.003$, $p_{TC} = 2.1 \times 10^{-6}$, $p_{TG} = 3.4 \times 10^{-8}$). Neither the measured plasma lipids, nor the GBS, were associated with histological features of atherosclerotic plaque composition. In addition, neither the plasma lipids nor the GBS were associated with clinical endpoints within 3 years of follow-up, with the notable exception of a negative association between HDL-C and composite cardiovascular endpoints.

Conclusion: This study found no evidence that plasma lipid levels or their genetic determinants influence carotid plaque composition.

1. Introduction

There is a wealth of evidence that plasma lipid levels influence the risk of cardiovascular disease (CVD). Most notably, Mendelian randomization studies have contributed unambiguous support for a causal effect of LDL-C and TG on CVD, while the evidence for the causality of HDL-C is perhaps not as clear [1–6]. Yet, for all the accumulated evidence on plasma lipids, the biological mechanisms by which they increase CVD risk are still incompletely understood. The traditional paradigm of atherosclerosis has been the accumulation of plasma lipids in the vascular wall contributing to the formation of an unstable plaque, the rupture of which causes a cardiovascular event. Much research has been performed in animal models and in vitro studies, but little is known about changes to

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human atherosclerotic plaque composition due to changed plasma lipid concentrations.

In addition to known determinants of plasma lipid levels such as diet, metabolism and body weight, it is clear that there is a heritable polygenic basis for inter-individual variation in lipid levels [7]. A large meta-analysis of genome-wide association studies by the Global Lipids Genetics Consortium (GLGC) identified 157 common single-nucleotide polymorphisms (SNPs) associated with high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglycerides (TG) blood concentrations [8]. Although the individual effects of these SNPs on plasma lipid concentrations are modest, the cumulative impact of multiple variants may be substantial. Since these SNPs confer a lifelong effect, they provide a useful tool to investigate the possible effects of plasma lipid levels on atherosclerotic plaque composition.

The Athero–Express Biobank Study (AE) has enrolled patients who underwent carotid endarterectomy (CEA) followed by extensive histological analysis of carotid plaque characteristics, genotyping and a 3 year follow-up for clinical endpoints. We investigated the association of plasma lipid concentrations with 7 features of atherosclerotic plaque composition in the Athero-Express. Subsequently, we performed similar associations using weighted genetic burden scores (GBS) to assess the cumulative effect of the known 157 lipid associated SNPs on plaque composition. In addition, we investigated if plasma lipids, or their genetic determinants, were associated with clinical endpoints during follow-up.

2. Methods

2.1. Inclusion

The Athero-Express Biobank Study is an ongoing multi-center cohort study including patients that underwent carotid endarterectomy and was previously described in more detail [9,10]. In short, blood and atherosclerotic plaques were obtained during surgery and stored at −80 °C. Clinical data were obtained through standardized questionnaires, pre-operative admission charts and patient medical files. Medical ethics committees of both hospitals approved the study and all patients included in the study provided informed consent.

2.2. Plaque histology

Atherosclerotic plaque specimens were collected during carotid endarterectomy, processed and analyzed according to a standardized and previously reported protocol [9]. In short, the specimen was paraffin-embedded, the culprit lesion was identified and a 5 micron cross-section was sliced, stained and quantified by microscopy. Hematoxylin and eosin (H&E) staining was used for assessment of calcifications and atheroma. Staining with Elastin von Gieson (EVG) was used for plaque hemorrhage and Picrosirius Red for assessment of collagen. Immunohistochemical staining was performed for assessment of macrophages (anti-CD68), microvessels (anti-CD34) and smooth-muscle cells (anti-alpha smooth muscle actin). The presence of calcifications and collagen were classified as scarce/absent or high. Atheroma was classified as below of above 10% of the plaque area. Plaque hemorrhage was classified as present or absent. Plaque microvessels were quantified by number of individual vessels per microscopy field. Plaque smooth-muscle cells and macrophages were quantified as percentage of the microscopy field area. For quantitative measures, multiple random microscopy fields were digitized, quantified and averaged.

2.3. Laboratory measurements

If routine clinical plasma lipids measurements were performed prior to surgery, that data was used. If available, stored pre-operative plasma samples were used for additional plasma lipids measurements at the clinical chemistry laboratory of the University Medical Center Utrecht. If both routine and additional lipids values were available the values were averaged (correlations routine vs. additional measurements: Spearman's $\rho > 0.94$ for all lipid traits).

2.4. SNP genotyping and imputation

Details on genotyping, quality control and imputation have been published previously [11]. In brief, DNA was extracted from whole blood, or alternatively from plaque samples, following standardized in-house validated protocols. Genotyping was done using commercially available genotyping chips. The first batch (Athero-Express Genomics Study 1, AEGS1) was genotyped using Affymetrix Genome-Wide Human SNP Array 5.0, the second batch (Athero-Express Genomics Study 2, AEGS2) was genotyped using the Affymetrix Axiom® GW CEU 1 Array (Affymetrix, Santa Clara, CA, USA). We adhered to community standard quality control and assurance (QCA) procedures to clean the genotype data obtained in AEGS1 ($N = 571$) and AEGS2 ($N = 868$) [12]. We used the 998 phased haplotypes from the Genome of the Netherlands Project release 4 (GoNL4) encompassing 19,763,454 SNPs as the reference panel for imputation [13].

2.5. Weighted genetic burden scores

The Global Lipids Genetics Consortium identified 157 loci associated with circulating lipid levels, and reported their effect sizes on plasma lipids (included in online supplement). We calculated weighted genetic burden scores using the risk alleles from these SNPs and their respective effect sizes. To account for the imputation quality we used the risk allele dosages to construct the GBS. The following formula was used to calculate the GBS for each lipid [14–16].

$$GBS_{lipid} = \Sigma(\beta_n * D_n)$$

where an individual's GBS_{lipid} is the sum of the effect size (β) of the risk allele of the nth SNP multiplied by the individual's dosage (D) of the risk allele for the nth SNP.

2.6. Follow-up and clinical outcome

Clinical follow-up was performed by contacting patients to fill-in standardized questionnaires at 1, 2 and 3 years after surgery. In case no response to the questionnaire was received the general practitioner was contacted for information. All events were assessed by two members of an outcome assessment committee. Cardiovascular death (CV-death) was defined as death of presumed vascular origin (stroke, myocardial infarction, sudden death, other vascular causes). Composite (cardiovascular) endpoints were defined as the occurrence of any cardiovascular event including CV-death, non-fatal stroke or myocardial infarction, or any vascular intervention not planned at the time of inclusion. Restenosis was determined based on routine clinical assessment of ipsilateral carotid artery stenosis using duplex echocardiography at 1 year post carotid endarterectomy.

2.7. Statistics

Statistical analysis was performed in RStudio using R (v3.1.2).

Regression analysis was performed using an additive linear model for continuous traits and a logistic model for categorical traits correcting for age, sex, hospital of surgery, diabetes or statin use, a dummy-variable representing the genotyping batch (AEGS1 or AEGS2), and the first 10 principal components. Prior to analysis, outliers at more than three standard deviations were removed from the data and traits that were not normally distributed (smooth-muscle cells, macrophages, microvessels) were normalized using Box-Cox transformation. We used PLINK [17] (version 1.7) to determine the associations of individual SNPs.

3. Results

3.1. Cohort characteristics

Clinical cohort characteristics are summarized in Table 1. The Athero-Express cohort is comprised of patients with advanced stages of atherosclerosis, as is evident from a high prevalence of atherosclerotic disease in various vascular territories (cerebrovascular accident 82.3%, coronary artery disease 29.9%, peripheral artery occlusive disease 20.7%). Associations of individual plasma lipids and GBS with risk factors are shown in Supplemental Tables 1 and 2

3.2. GBS associate with circulating lipids

To ascertain the validity of the calculated GBS as a means to investigate the relationship between plasma lipids and CVD, we first confirmed the relationship between GBS and measured plasma lipids. All the GBS showed significant associations with their respective plasma lipid concentrations ($p_{HDL\text{-}C} = 2.4 \times 10^{-14}$, $p_{LDL\text{-}C} = 0.003$, $p_{TC} = 2.1 \times 10^{-6}$, $p_{TG} = 3.4 \times 10^{-8}$) and corrected plasma lipids showed an increasing trend with higher GBS for all lipid traits (Fig. 1A–D). This replicates previous studies, and confirms the effects of these SNPs on plasma lipids in this cohort of patients with severe atherosclerotic disease. Remarkably, there was only a moderate association between the LDL burden score and measured LDL-C levels, which may be explained by statin-induced favorable LDL-C levels, irrespective of the genetic burden (Suppl. Fig. 1). Additionally, we investigated all SNPs individually for their association with each of the plasma lipids (Supplemental Table 3), yet none of the SNPs were significant after Bonferroni correction.

Table 1
Cohort characteristics.

Characteristic	Median (IQR)	Missing (%)
Age, years	69.9 (62.0–76.0)	0.0
SBP, mmHg	153 (138–170)	16.6
DBP, mmHg	80 (74–90)	16.6
BMI, kg/m [2]	25.9 (24.0–28.4)	6.9
GFR, ml/min/1.73 m [2]	72.2 (58.9–85.1)	3.5
hsCRP, mg/l	1.97 (0.92–4.41)	38.9
HDL-C, mmol/l	1.11 (0.90–1.37)	36.2
LDL-C, mmol/l	2.70 (2.02–3.39)	39.7
TC, mmol/l	4.63 (3.83–5.46)	33.3
TG, mmol/l	1.39 (1.00–1.96)	36.5
Smooth-muscle cells,#/field	1.34 (0.50–2.78)	2.9
Macrophages,#/field	0.34 (0.08–1.047)	4.2
Microvessels,#/field	7.0 (4.0–11.3)	10.1
	Percentage	Missing (%)
Gender, male	67.8	0.0
Smoking	24.3	2.3
Statin	76.3	0.1
Antiplatelets	88.7	0.3
Diabetes	23.0	0.0
Hypertension	85.6	0.1
CAD	29.9	0.1
MI	19.3	1.1
Stroke	33.1	0.0
CVA	82.3	0.0
PAOD	20.7	0.1
Calcification, high	50.2	1.9
Collagen, high	80.3	1.8
Atheroma, >10%	72.5	1.7
Plaque hemorrhage, present	60.4	1.8

Clinical characteristics of all patients at the time of study inclusion. Patient history of CAD, MI, Stroke, CVA and PAOD were scored as percentage of cases prior to inclusion. hsCRP, smooth-muscle cells, macrophages and microvessels are presented as non-transformed data. IQR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body-mass index; eGFR, estimated glomerular filtration rate (MDRD formula); hsCRP, high-sensitive C-reactive protein; CAD, coronary artery disease; MI, myocardial infarction; CVA, cerebrovascular accident; PAOD, peripheral artery occlusive disease.

3.3. Associations with plaque characteristics

We proceeded to investigate if measured plasma lipid levels were associated with histological features of atherosclerotic plaque composition. Plaque features were determined quantitatively (number of smooth-muscle cells, macrophages, and microvessels) or semi-quantitatively (for crude levels of collagen, calcification, plaque hemorrhage, and atheroma), and associated with lipid levels. We observed nominally significant associations for HDL-C with fewer plaque macrophages ($p = 1.6 \times 10^{-3}$) as well as for LDL-C ($p = 2.0 \times 10^{-2}$), TC ($p = 8.2 \times 10^{-3}$) and TG ($p = 1.4 \times 10^{-2}$) with less plaque calcification (Table 2). Similarly, we investigated if GBS of lipids were associated with histological features of atherosclerotic plaque composition. Nominally significant associations were observed for both LDL-scores ($p = 2.6 \times 10^{-2}$) and TC-scores ($p = 2.0 \times 10^{-2}$) with atheroma size (Table 2). After Bonferroni correction however, none of the plasma lipids and none of the GBS were significantly associated with histological plaque features. Since the use of statins was high in this cohort (76.3%) and strongly associated with plasma LDL-C ($p = 8.9 \times 10^{-20}$) and plasma TC concentrations ($p = 2.8 \times 10^{-15}$) (Supplemental Table 1), we also performed a subgroup analysis in the 344 patients not using statins (Supplemental Table 4). This showed generally similar results, with the exception of stronger negative associations with plaque calcification for the LDL-burden scores ($p = 6.5 \times 10^{-3}$) and TC-burden scores ($p = 7.6 \times 10^{-3}$), although these were not significant after Bonferroni correction. Additionally, we investigated all SNPs individually for their association with plaque characteristics, but none reached statistical significance after Bonferroni correction (Supplemental Table 3).

3.4. Associations with clinical endpoints

Despite the lack of significant associations with plaque features, we investigated whether plasma lipids and their corresponding GBS were associated with the incidence of cardiovascular endpoints within 3-years of follow-up. Increased HDL-C levels were significantly associated with a reduction of composite cardiovascular endpoints, also after correction for multiple testing, whereas none of the GBS were significantly associated (Table 3). In addition, we found no significant associations between individual SNPs and clinical endpoints, after correction for multiple testing (Supplemental Table 3).

4. Discussion

To our knowledge, this study is the first effort to associate plasma lipids and genetic factors influencing them, with histological features of carotid plaques, which are widely considered to be surrogates of cardiovascular disease severity and risk [10,18,19]. We

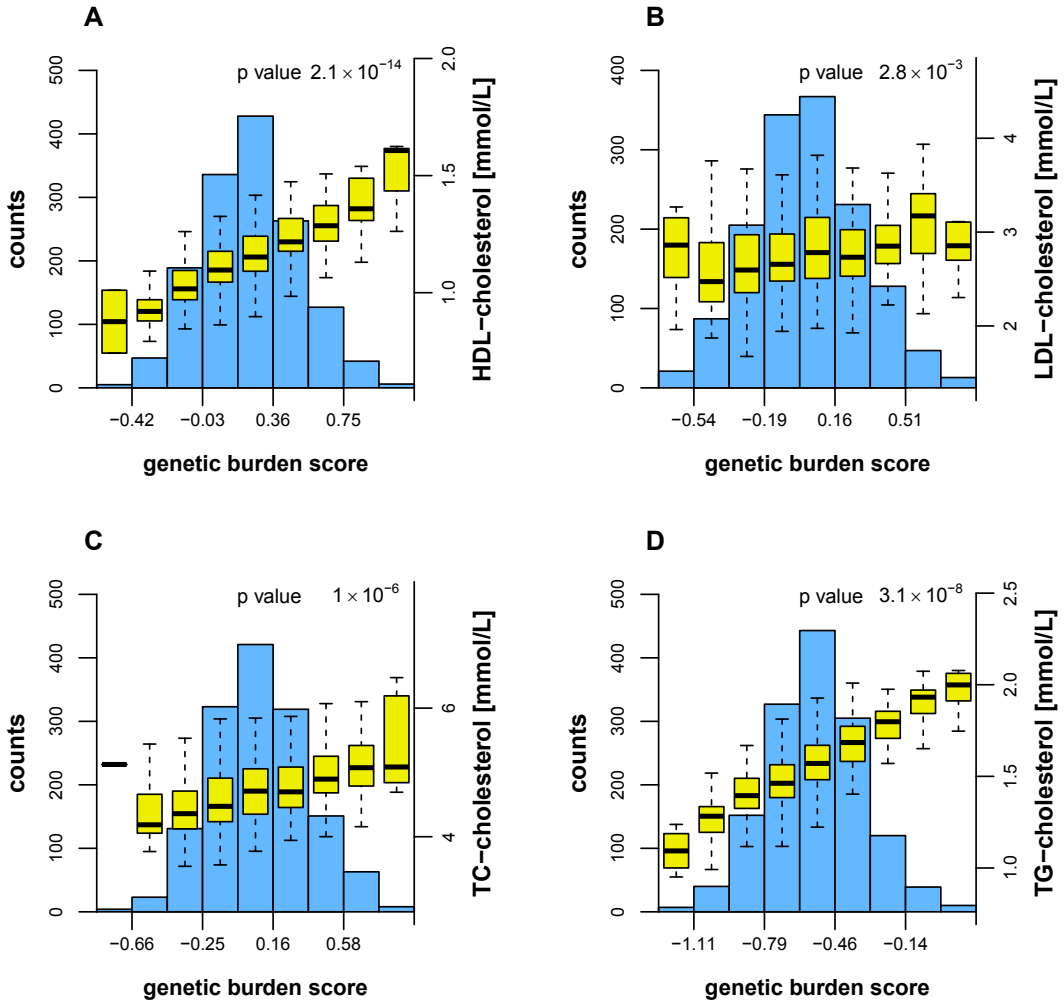

Fig. 1. Relationships between plasma lipids and corresponding genetic burden scores. Combined histograms-boxplots showing the model relationship between genetic burden scores and plasma lipids using linear regression modeling. Histograms show distribution (left axis) of the genetic burden score (x axis) in the cohort population. Boxplots show corrected plasma lipid concentrations (right axis), i.e. model fitted values, within the respective histogram bin. Significance of the (continues) association between genetic burden score and the respective plasma lipid denoted as p-value. A) HDL-C; B) LDL-C; C) Total TC; D) TG.

show that neither plasma lipids nor genetic burden scores of plasma lipids are associated with carotid atherosclerotic plaque composition. This may seem somewhat surprising in light of the current paradigm of the "vulnerable plaque". Given the overwhelming evidence of LDL-C as a causal contributor to CVD risk, it is perhaps remarkable that no effects on plaque composition were found.

Several points are worth highlighting and may explain the lack of association of plasma lipids or the GBS with plaque features. (i) We performed this study in a patient cohort of limited size, which lacked well-matched healthy subjects as controls as it is unfeasible to obtain carotid plaque samples from healthy subjects. This may have limited biological variance in the observed plaque features. Both cohort size and lack of controls may limit the power to confidently prove a lack of associations, particularly if the magnitude of effect is small. (ii) The patients in this cohort had severe atherosclerotic disease and many of them suffered from comorbidities with effects we may not have been able to fully account for in the analysis. (iii) The majority of patients received lipid altering medications, which may have variable effects on individual lipid level improvement. This is something we cannot correct for in the model, and may explain the relatively weak association of the LDL-burden score with the measured LDL plasma concentrations in these patients, especially when compared to the other lipid fractions. The high use of lipid-lowering medications may also have improved plaque composition, and may have prevented cardiovascular events in some patients. This could mask the effects of lipids or the GBS, although sub-analysis in non-statin users showed generally similar results. (iv) We have shown that carotid plaque histological examination is prone to some inter-observer bias [20]. This is due to a certain level of subjective interpretation by the observer, affecting the precision to determine plaque composition. (v) With regard to plasma lipids, we were only able to include a pre-operative measurement, while plasma lipid concentrations may vary considerably over time. (vi) Also, we suffered from the incomplete availability of plasma lipid concentrations (missingness 33.3%–39.7%), which may contribute to bias. (vii) For most SNPs previously associated with lipid levels, the mechanism of action is unknown. Conceivably, unknown factors may modify their effect on plasma lipids between individuals. This would also explain the different effect sizes when comparing our study to the GLGC study results.

An alternative explanation is that plasma lipid levels may have a negligible effect on carotid plaque composition, and may exert their effects on the risk of CVD through other mechanisms (for example, coronary calcification [21]). In accordance, a recent meta-analysis

Table 2
Associations of Plasma lipids and their genetic burden scores with plaque composition.

Lipid spectra	HDL-C		LDL-C		TC		TG	
	Beta (SE)	***P***	***Beta (SE)***	***P***	***Beta (SE)***	***P***	***Beta (SE)***	***P***
Smooth-muscle cells	−0.21 (0.13)	0.097	0.06 (0.05)	0.206	0.00 (0.04)	0.966	0.02 (0.06)	0.678
Macrophages	−0.39 (0.12)	0.002	0.03 (0.05)	0.598	−0.03 (0.04)	0.371	−0.02 (0.06)	0.726
Microvessels	0.04 (0.21)	0.843	−0.09 (0.08)	0.278	−0.03 (0.06)	0.666	0.18 (0.09)	0.052
	OR (95% CI)	***P***	***OR (95% CI)***	***P***	***OR (95% CI)***	***P***	***OR (95% CI)***	***P***
Calcification	1.08 (0.79–1.49)	0.678	0.84 (0.74–0.95)	0.020	0.85 (0.77–0.94)	0.008	0.81 (0.7–0.93)	0.014
Collagen	1.26 (0.84–1.89)	0.351	1.15 (0.98–1.34)	0.144	1.05 (0.94–1.19)	0.470	0.99 (0.83–1.19)	0.942
Atheroma	0.74 (0.52–1.06)	0.171	1.14 (0.99–1.32)	0.127	1.11 (0.99–1.24)	0.135	1.09 (0.93–1.3)	0.383
Plaque hemorrhage	0.89 (0.64–1.24)	0.553	1.00 (0.88–1.14)	0.996	1.03 (0.93–1.13)	0.687	1.10 (0.95–1.28)	0.298
Genetic Burden Scores	HDL-score		LDL-score		TC-score		TG-score	
	Beta (SE)	***P***	***Beta (SE)***	***P***	***Beta (SE)***	***P***	***Beta (SE)***	***P***
Smooth-muscle cells	−0.12 (0.13)	0.366	0.08 (0.13)	0.536	0.04 (0.13)	0.728	0.06 (0.16)	0.728
Macrophages	−0.06 (0.13)	0.667	0.18 (0.13)	0.174	0.08 (0.13)	0.530	0.15 (0.16)	0.334
Microvessels	−0.15 (0.22)	0.496	0.02 (0.22)	0.919	0.02 (0.22)	0.939	0.53 (0.27)	0.055
	OR (95% CI)	***P***	***OR (95% CI)***	***P***	***OR (95% CI)***	***P***	***OR (95% CI)***	***P***
Calcification	0.82 (0.58–1.15)	0.324	0.83 (0.6–1.16)	0.365	0.80 (0.57–1.11)	0.254	0.77 (0.51–1.16)	0.294
Collagen	0.84 (0.55–1.28)	0.503	1.47 (0.97–2.23)	0.125	1.35 (0.9–2.02)	0.228	1.52 (0.91–2.55)	0.183
Atheroma	0.94 (0.64–1.38)	0.799	1.67 (1.15–2.44)	0.026	1.69 (1.17–2.45)	0.020	1.49 (0.94–2.39)	0.159
Plaque hemorrhage	0.85 (0.6–1.21)	0.446	1.18 (0.84–1.67)	0.421	1.21 (0.86–1.7)	0.348	1.15 (0.75–1.78)	0.589

The association of plasma lipids and genetic burden scores with histological features of plaque composition. Smooth-muscle cells, macrophages and microvessels were scored quantitatively as the number of features per microscopy field. Quantitative histology features were normalized using Box-Cox transformation and associated to plasma lipids and GBS using linear regression modeling. Calcification, collagen, atheroma and plaque hemorrhage were determined semi-quantitatively. Plasma lipids and burden scores were associated to increased abundance of the semi-quantitative feature using logistic regression modeling. Results are given as model beta with standard error, or as odds ratios with 95% confidence interval.

Table 3
Associations of plasma lipids and their genetic burden scores with clinical endpoints.

	HDL-C		LDL-C		TC		TG	
	HR (95% CI)	p val	HR (95% CI)	p val	HR (95% CI)	p val	HR (95% CI)	p val
Restenosis[a]	1.23 (1.02–1.48)	7.0×10^{-2}	1.15 (0.93–1.41)	0.28	1.25 (1.04–1.52)	5.0×10^{-2}	0.92 (0.76–1.1)	0.46
Stroke	0.87 (0.67–1.14)	0.31	0.82 (0.62–1.09)	0.17	0.74 (0.57–0.97)	2.9×10^{-2}	1.01 (0.79–1.29)	0.95
Myocardial Infarction	0.75 (0.52–1.1)	0.14	1.08 (0.74–1.57)	0.68	1.11 (0.78–1.58)	0.55	1.12 (0.82–1.54)	0.46
Cardiovascular death	0.77 (0.53–1.1)	0.15	0.81 (0.54–1.19)	0.28	0.71 (0.49–1.01)	5.7×10^{-2}	1.18 (0.89–1.58)	0.25
Composite endpoints	0.74 (0.63–0.86)	1.1×10^{-4}	0.86 (0.74–1.01)	6.0×10^{-2}	0.86 (0.75–0.99)	3.6×10^{-2}	1.12 (0.99–1.27)	7.9×10^{-2}
	HDL-score	LDL-score		TC-score		TG-score		
	HR (95% CI)	p val	HR (95% CI)	p val	HR (95% CI)	p val	HR (95% CI)	p val
Restenosis[a]	1.02 (0.88–1.19)	0.81	1.05 (0.89–1.23)	0.63	1.04 (0.89–1.22)	0.66	0.99 (0.85–1.16)	0.94
Stroke	0.91 (0.74–1.12)	0.37	1.15 (0.94–1.41)	0.19	1.07 (0.87–1.31)	0.53	1.05 (0.86–1.29)	0.62
Myocardial Infarction	0.85 (0.65–1.11)	0.23	0.91 (0.69–1.2)	0.52	0.88 (0.67–1.15)	0.35	1.04 (0.8–1.36)	0.76
Cardiovascular death	0.91 (0.71–1.17)	0.46	0.97 (0.75–1.26)	0.80	0.93 (0.71–1.2)	0.56	1.18 (0.92–1.52)	0.18
Composite endpoints	0.94 (0.84–1.05)	0.27	1.03 (0.92–1.15)	0.61	0.98 (0.88–1.09)	0.74	0.98 (0.88–1.09)	0.72

Cox proportional hazard model associating plasma lipids and genetic burden scores with clinical endpoints within 3 years. Results are given as hazard ratio per standard deviation increase with 95% confidence intervals.

[a] Restenosis was assessed after 1 year by carotid echography as more than 50% stenosis of the vascular lumen, and was associated using logistic regression modeling, showing odds ratio (95% CI) instead of hazard ratio.

showed that statin treatment leads to carotid plaque regression through improvement of inflammation, not lipid levels [22].

We also investigated if plasma lipids or genetic variants were associated with clinical endpoints within 3 years, but failed to show an association. We also included myocardial infarction as an endpoint, as there is a partially shared etiology [23] and patients with carotid artery disease are at increased risk of cardiovascular disease in other vascular territories [24]. The notable exception is a negative association between HDL-C levels and the occurrence of composite cardiovascular endpoints, which may represent a non-causal effect (e.g. due to reverse causation or uncorrected confounding), consistent with recent Mendelian randomization studies [2,4,5]. The aforementioned limitations as well as the limited follow-up period of 3 years in this actively monitored and treated patient group may have decreased power to show an effect.

Replication at present seems challenging, as we are unaware of cohorts that possess similar data with adequate sample size. Future efforts in larger samples will have better power to address the causal role of lipid fractions and the “vulnerable plaque” in cardiovascular disease. If it is true that there is no relation between lipid fractions and plaque features, then that would genuinely question the mechanisms by which plasma lipids contribute to CVD risk, as well as the paradigm of the vulnerable plaque, yet we do not believe our current results are sufficiently robust to arrive at that conclusion.

In summary, this study found no evidence that plasma lipid levels or genetic determinants of plasma lipid levels influence carotid plaque composition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.07.041.

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